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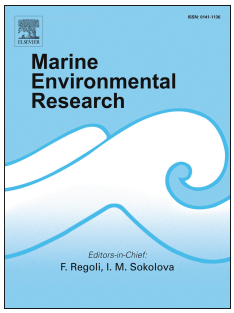

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Drivers of phytoplankton production and community structure in nutrient-poor estuaries receiving terrestrial organic inflow

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Abstract

The influence of nutrient availability and light conditions on phytoplankton size-structure, nutritional strategy and production were studied in a phosphorus-poor estuary in the northern Baltic Sea receiving humic-rich river water. The relative biomass of mixotrophic nanophytoplankton peaked in spring when heterotrophic bacterial production was high, while autotrophic microphytoplankton had their maximum in summer when primary production displayed highest values. Limiting substance only showed small changes over time, and the day light was at saturating levels all through the study period. We also investigated if the phytoplankton taxonomic richness influences the production. Structured equation modelling indicated that an increase of the taxonomic richness during the warm summer combined with slightly higher phosphorus concentration lead to increased resource use efficiency, which in turn caused higher phytoplankton biomass and primary production. Our results suggest that climate warming would lead to higher primary production in northerly shallow coastal areas, which are influenced by humic-rich river run-off from un-disturbed terrestrial systems.

Keywords

Phytoplankton, Size-structure, Primary production, Autotrophy, Mixotrophy, Taxonomic richness, Resource use efficiency, Coastal waters, Phosphorus-poor estuaries,

Highlights:

- Mixotrophic nanophytoplankton is influenced by spring river flush and bacterial production.
- Phytoplankton diversity and resource use efficiency are promoted by summer warming and phosphorus concentration.
- High resource use efficiency sustains phytoplankton biomass and primary production.
- Autotrophic microphytoplankton is favored during the summer primary production maximum.

Introduction

Phytoplankton communities are governed by many limiting and controlling factors, such as nutrient availability, light climate, temperature, salinity, competition, parasites and grazing (Andersson et al., 1996; Calbet, 2001; Dahlgren et al., 2010; Faithfull et al., 2011). In temperate aquatic systems the phytoplankton succession generally starts with a spring bloom dominated by relatively large autotrophic cells, which are favored by high light and nutrient concentrations. As nutrients are depleted and the water warms up, smaller plankton, i.e. autotrophic, mixotrophic and heterotrophic nano- and picoplankton are promoted (Sommer et al., 1986; Andersson et al., 1996; Legrand et al., 2015). These have a competitive advantage at low nutrient conditions due to their high surface to volume (S/V) ratio and thinner diffusion boundary layer (Raven, 1998). However, in systems (e.g. lakes) influenced by terrestrial dissolved organic matter (tDOM), the plankton succession pattern can be reversed. A profusion of tDOM during spring can decrease light availability and promote the growth of heterotrophic bacteria resulting in a weakened or absent phytoplankton bloom, with maximum primary production rates occurring instead during the warmer summer months when river discharge is lower (Drakare et al., 2002; Figueroa et al., 2016). Under such conditions filamentous cyanobacteria could be promoted due to their capacity for phosphorus storage, atmospheric nitrogen fixation and buoyancy regulation (Paerl and Paul, 2012; Reynolds, 2006). However, the drivers of coastal phytoplankton communities are complex and may depend on the relative influence of river inflow and hydrodynamic interaction with offshore waters.

Many estuaries are highly productive, as phytoplankton growth is nurtured by river borne nutrients (Dorado et al., 2015; O'Boyle and Silke, 2010). However, rivers not only transport nutrients, but also tDOM, including coloured tDOM, to the sea. Studies have shown that tDOM can have both a positive and a negative effect on primary production (Andersson et al., 2013; Thrane et al., 2014; Seekell et al., 2015). High concentrations of tDOM can limit primary production by absorbing light and reducing the availability of phosphorus and iron, essential factors for phytoplankton growth (Jones, 1992; Carpenter et al., 1998). However, the promotion of phytoplankton growth due to the shielding effect from harmful ultraviolet (UV) radiation, and transportation of bioavailable nutrients, can also occur (Nielsen and Ekelund, 1993; Kissman et al., 2013; Seekell et al., 2015).

Since tDOM incorporates potentially bioavailable carbon, heterotrophic bacteria and the heterotrophic microbial food web can be favored (Tranvik, 1989; Jansson et al., 2007; Barrera-Alba et al., 2009; Hitchcock and Mitrovic, 2015). Under conditions favoring bacterial growth and production, a higher contribution of potentially mixotrophic flagellates has also been observed, which is explained by their ability to supplement photoautotrophic processes by ingesting bacteria (Jansson 1996; Bergström et al., 2003; Stoecker et al., 2017). Consequently, the negative impacts of tDOM on phytoplankton growth and the concurrent promotion of heterotrophic bacteria can alter the ecosystem productivity and trophic balance (Sandberg et al., 2004; Andersson et al., 2013). Furthermore, this may affect the size-structure of the food web, which has implications for the number of trophic levels and the food web efficiency (Legendre and Rassoulzadegan, 1995; Havens, 1998; Dahlgren et al., 2010).

A less studied factor that may influence aquatic productivity is taxonomic richness. A few previous studies indicate that productivity and diversity display a unimodal or positive relationship (Irigoien et al., 2004; Korhonen et al., 2011). In unimodal relationship, at low

productivity low resource availability would limit the number of species, while at high productivity, the phytoplankton community is dominated by few highly competitive species (Rosenzweig and Abramsky, 1993). An increase in productivity due to higher diversity is explained by higher possibility to contain more productive species (selection effect) as well as species which are complementary in the use of resources (complementarity effects) (Loreau at al., 2001; Loreau at al., 2002). The diversity-productivity relationship is likely to occur both on the geographical (e.g. local vs. regional) and ecological scale (e.g. within vs. between communities) (Waide et al., 1999; Gross at al., 2000). Highly diverse communities may occupy more niches than communities with lower diversity, which in turn might result in higher resource use efficiency (Loreau at al., 2001; Loreau at al., 2002). However, while some studies show a positive correlation between diversity and phytoplankton resource use efficiency (RUE) (Ptacnik et al., 2008), others do not (Hodapp et al., 2015). Thus, there is need for more studies to get a general understanding of the relationship between taxonomic richness, resource use efficiency and productivity in different ecosystems.

The northern Baltic Sea is strongly influenced by phosphorus-poor riverine inflows with high tDOM concentrations (Kuklinski and Pempkowiak, 2011; Pettersson et al., 1997). In the north tDOM makes up ~80% of the dissolved organic matter pool (Alling et al., 2008). Seasonal variations in river discharge and characteristic differences in the properties of the catchment areas are major factors affecting the supply of organic matter to the sea (Skoog et al., 2011; Asmala et al., 2013). The inflow of tDOM to coastal areas is highest during spring when snowmelt in forest and peatland dominated areas takes place (Pettersson et al., 1997; Räike et al., 2012; Reader et al., 2014). Climate scenarios indicate that precipitation and thus the inflow of tDOM to the northern Baltic Sea will increase in the future (Meier, 2006).This has the potential to modify the composition and size-structure of the phytoplankton community as well as alter the ratio between primary and bacteria production, with potential consequences for higher trophic levels.

Effects of increasing tDOM to coastal ecosystems, and thus surface water browning, due to climate change may also increase the release of greenhouses gases to the atmosphere (e.g., CO_2, N_2O) as a consequence of shift from net autotrophic to net heterotrophic ecosystem (Wikner and Andersson, 2012; Lapierre et al., 2013). Additionally, heterotrophic bacteria as well as cyanobacteria are considered to be lower food quality for consumers because of lack of polyunsaturated fatty acids (PUFAs) in their cells, which can decrease food web efficiency of the system (Harwood and Russell, 1984; Berglund et al., 2007). Higher tDOM concentrations may also lead to decreased light reaching benthic environment, decreased oxygen production by autotrophs and increased coastal dead zones (Jones, 1992; Andersson et al., 2015). Changes in tDOM input, stronger stratification and a decreased photic zone may promote filamentous cyanobacterial growth due to their capability for phosphorus storage, atmospheric nitrogen fixation and buoyancy regulation (Ibelings et al., 1991; Pettersson et al., 1993).

The aim of this study was to elucidate factors governing the production, size-structure and nutritional strategy in the phytoplankton community in a sub-arctic estuary with low nutrient concentrations and exposure to seasonal river discharge. We tested 1) what factors are influencing phytoplankton size-structure and production 2) if mixotrophic nanophytoplankton is promoted by heterotrophic bacterial production, and 3) if phytoplankton taxonomic richness is positively correlated to resource use efficiency (RUE) and phytoplankton production. Our results contribute to the understanding of the structure and function of phytoplankton communities in ecosystems heavily influenced by tDOM,

and give insights into the potential ecological consequences of climate change in coastal environments.

Material and Methods

Field sampling

The study was performed in the sub-arctic Råne estuary, northern Baltic Sea, Sweden (Fig. 1). Monthly sampling was performed at 19 stations from May to August (2011) to encompass the river and its discharge area within the estuary. Henceforth May is referred to as spring while the remaining months are referred to as summer. Station 1 was located at the river mouth while 18 stations were evenly dispersed across the estuary region, with the most seaward station being circa 10 km from the river station sampled (Fig. 1). Water was collected at a depth of 1 m using a Ruttner sampler and transported to the laboratory in shaded 20 l bottles. Additional samples for primary and bacterial production were also taken at 3 and 5 meters (where depth allowed) to determinate depth-integrated production. Temperature (Temp) and Photosynthetically active radiation (PAR) were measured *in situ*. Underwater PAR was recorded every 50 cm in the water column with a Licor LI-1400 connected to Spherical SPQ 1730 sensor, and surface incident PAR was monitored at the Umeå Centre for Marine Sciences (Licor LI-193 spherical quantum sensor). Light attenuation coefficient (Kd) was estimated from the slope of the linear regression of the natural logarithm of down-welling irradiance versus depth. Underwater PAR values recorded at each station (in total 19 stations), were used to calculate average PAR at 1 and 5 m depth for the specific sampling event each month.

Conductivity and pH were measured at 25°C (Mettler Toledo probes) and *in situ* values were obtained according to Fofonoff and Millard (1983). Salinity was calculated from measurements of *in situ* conductivity. Samples for total nitrogen (Tot N), total phosphorus (Tot P), humic substances (HS), dissolved organic carbon (DOC), coloured dissolved organic matter (CDOM), chlorophyll *a* (Chl *a*), suspended particulate matter (SPM), phytoplankton species composition and biomass were preserved immediately on arrival to the laboratory. Data on river water discharge were obtained from the Swedish Meteorological and Hydrological Institute (SMHI).

Physicochemical analyses

Tot P and Tot N were measured in unfiltered water samples using a Braan and Luebbe TRAACS 800 autoanalyzer, according to standard analytical methods (Grasshoff et al., 1983). Tot P and Tot N were considered to reflect the nutrients available to the phytoplankton. This assumption is based on results obtained in previous studies in the northern part of the Baltic Sea, during which a positive correlation with inorganic form was found. DOC analysis was performed on 0.22 µm filtered (Supor Membrane Syringe Filter, non-pyrogenic; Acrodisc®) and acidified water (18 mM HCl, final concentration). Samples were analyzed on a Shimadzu TOC-5000 analyzer. Measurements of Tot P, Tot N and DOC were performed at an accredited laboratory at Umeå Marine Sciences Center (UMF). Humic substances (HS) were determined from unfiltered water samples using a Perkin Elmer LS 30 fluorometer at 350/450 excitation/emission wavelengths. Calibration standards were prepared from quinine dihydrogen sulfate dehydrate in 0.05 M sulfuric acid (Hoge et al., 1993; Wedborg et al., 1994). Sulfuric acid (0.05 M) was used as blank.

CDOM absorbance was measured in water samples filtered through a 0.22 µm polycarbonate membrane and stored in amber glass bottles in the dark at 4°C until analysis. Absorbance values were recorded from 250 to 800 nm using Shimadzu UVPC-2501 scanning spectrophotometer, with Mili-Q water as the blank. The absorption coefficient at 440 nm was calculated by multiplying the absorbance at specific wavelength with 2.303 and divided by the length of the cuvette (Kirk, 2011).

SPM was measured using the gravimetric method described by Strickland and Parsons (1972). Triplicate 1 l water samples were filtrated through pre-combusted (450°C) and pre-weighed (W_0) Whatman GF/F filters. Post-sampling, filters were dried for 24 hours at 60°C and re-weighed (W_1). The final concentration of SPM was calculated as the average of triplicates (W_1 - W_0).

Chlorophyll *a* and primary production

Samples for Chl *a* (100 ml) were filtrated onto 25 mm GF/F filters under low pressure and stored at -80°C until analysis. Chl *a* was extracted in 95% ethanol in the dark overnight at 4ºC. Samples were centrifuged for 10 minutes to separate ethanol containing chlorophyll *a* from solid material. The concentration of chlorophyll *a* was measured with a Perkin Elmer LS 30 fluorometer (433 nm excitation and 674 nm emission wavelength).

In situ photosynthetic rates of phytoplankton were measured using the ^{14}C incorporation method. 5 ml of seawater were placed in four 20 ml bottles (three light and one dark) and incubated *in situ* with 7.2 µl ^{14}C (^{14}C Centralen Denmark, activity 100 µCi ml^{-1}) for a minimum of 3 hours. Post incubation, 100 µl of 5 M hydrochloric acid were added to each tube and samples were ventilated for 12 hours. 15 ml of scintillation cocktail were added to each sample and samples were measured on a Beckman 6500 scintillation counter. Dissolved inorganic carbon was calculated based on temperature, pH and salinity according to Gargas (1975). Daily net primary production (PP) was calculated using the "light factor method" as described in Gargas (1975) and Andersson et al. (1996).

Bacterial production

The ^{3}H-thymidine incorporation method was used to measure bacterial production (BP) (Fuhrman and Azam, 1982). Triplicate 1 ml seawater samples (one control and two samples) were incubated with 2 µl of ^{3}H-thymidine (84 Ci $mmol^{-1}$; PerkinElmer, Massachusetts, USA) (final concentration 24 nM) for 1 hour at *in situ* temperature. This thymidine addition corresponded to the saturation level. The control sample was pre-killed by adding 100 µl of ice-cold 50 % TCA and incubation at -20°C for 5 minutes. Cell production was calculated using a conversion factor of 1.4×10^{18} cells mol^{-1} of incorporated thymidine (Wikner and Hagström, 1999). Daily net production rates were calculated assuming stable uptake rates over the day and a bacterial carbon content of 20 fgC $cell^{-1}$ (Lee and Fuhrman, 1987). The assumptions are based on diel experiments and measurements and bacterial cell sizes in the study area (data not shown).

Plankton identification and enumeration

Samples for analysis of nano- and microplankton were fixed with 2% acidic Lugol's solution. 10-50 ml samples were settled for 12-48 hours in sedimentation chambers. The cells were then counted with an inverted microscope using phase contrast imaging (Nikon Eclipse Ti) (Utermöhl, 1958). Microplankton (> 20 µm) and nanoplankton (2-20 µm) samples were counted at 100x and 400x magnification, respectively. For ciliates, 200x

magnification was used. Different taxa and their nutritional characteristics were identified from the cell morphology, size and described trophy (Tikkanen and Willen, 1992; Hällfors, 2004; Olenina et al., 2006). Further, the coloration of the smallest cells was used to support the trophy classification as Lugol's solution stains chlorophyll *a* brown. Cell biovolume of autotrophic, heterotrophic and mixotrophic plankton and the ciliate (*Mesodinium rubrum*) were calculated according to Olenina et al. (2006) and carbon content was estimated following the Menden-Deuer and Lessard equations (Menden-Deuer and Lessard, 2000).

Picocyanobacteria were analyzed using epifluorescence microscopy, as described in Andersson et al. (1996). The samples were preserved with glutaraldehyde (2% final concentration), filtered (1 ml) onto 0.6 µm black polycarbonate filters and counted on an epifluorescence microscope (Nikon Eclipse TE 2000-U) at 1000x magnification, using green excitation light (510 – 560 nm, emission wavelength > 590 nm). Cells were counted in 20 randomly positioned fields of view, and a minimum of 300 cells were counted per sample. Biovolume and carbon biomass were estimated as described above.

Cells were grouped into three functional groups (AU: autotrophs, HT: heterotrophs, MX: mixotrophs), and three size categories (picoplankton: < 2 µm, nanoplankton: 2–20 µm, microplankton: > 20 µm), based on measurements of the longest cell axis. Total phytoplankton biomass (TB) was calculated as the sum of the carbon biomass of autotrophs (including *Mesodinium rubrum*) and mixotrophs. The relative biomass proportion of functional groups and size classes was calculated.

Phytoplankton taxonomic richness (S), defined as the number of taxa found in a sample was calculated as a proxy of diversity. Phosphorus has been shown to be the main limiting factor for phytoplankton in the studied coastal area in the northern Baltic Sea (Andersson et al., 1996), and therefore the phytoplankton resource use efficiency (RUE) was expressed as a natural logarithm of the ratio between TB and Tot P (RUE_P) (Ptacnik et al., 2008).

Statistical analyses

Generalized linear mixed-effects model (GLMM) was used to identify relationships between biological and physicochemical variables. Based on variance inflation factor (VIF) results, HS was not included in analysis due to high multicollinearity with other parameters e.g. DOC (VIF>10). A backwards stepwise elimination process based on Akaike Information Criterion (AICs) was used to remove nonsignificant variables and obtain the final model. Additionally, Spearman's correlation coefficients were calculated between phytoplankton related variables and physicochemical variables. Changes in the phytoplankton composition between months and stations were visualized by non-metric multidimensional scaling (NMDS) based on Bray-Curtis similarity matrix, while analysis of similarity (ANOSIM) was performed to test differences in phytoplankton biomass composition between months. Phytoplankton abundance for both analyses was standardized by sample size. The redundancy analysis (RDA) was conducted to identify main physicochemical and biological variables influencing the size-structure of the phytoplankton. As the results of the variance inflation factor (VIF) indicated that HS was highly correlated with other variables, this variable was excluded from the RDA analysis. Pearson's correlation (r_p) between variables was estimated by RDAs. Forward selection (and a Monte-Carlo permutation test, n = 999 permutations) was used to estimate which variables had significant influence on size-structure of the phytoplankton. Additionally, relationships between phytoplankton taxonomic richness (S), resource use efficiency (RUEp), total biomass (TB) and primary production (PP) as endogenous variables and total

phosphorus (Tot P) and temperature (Temp) as exogenous variable were examined by piecewise structural equation models (piecewiseSEMs). We used the d-separation (d-sep) test to investigate if all pathways in the model were included. Unstandardized path coefficients and R^2 values were calculated, while Fisher's test was used to investigate goodness of fit of the model. Data analyses were performed in R version 3.5.1 using the package 'MASS', 'piecewiseSEM', SPSS Statistics 22, Primer 6 and Canoco 5 softwares.

Results

Physicochemical variables

The river water discharge was highest during the May sampling, with flow rates of ~100 $m^3 s^{-1}$, after which it was lower, ~30 $m^3 s^{-1}$ for the remainder of the study period (Fig. S1, Table 1). Salinity increased from ~ 0.3 to 1 over time due to reduced river inflow (Table 1). Water temperature was < 8°C in May and increased to > 15°C in summer, reaching highest values in July (Table 1, Fig. 2D). Tot N and DOC concentrations were highest in May then decreased, and stayed at a similar level during summer (Table 1). A similar temporal trend was also observed for Kd (Table 1). Average Tot P was lowest in May and slightly increased during the remaining months (Table 1, Fig. 2 E). The ratio between Tot N and Tot P was highest in May, ~90, then decreased and stayed the same for the remaining months (Table 1). SPM and pH increased from May to August (Table 1). tDOM related variables, such as humic substances and CDOM showed highest values in May and July, concomitant to the river flush (Table 1). Generally, higher values were observed close to the river mouth and lesser at the more seawater locations (data not shown). The lowest average PAR at 1 m was observed in June and the highest in July while remaining at a level of ~ 100 µmol photon $m^{-2}s^{-1}$ during the other months (Table 1, Fig. 2F). PAR at 5 m was only about 5-10% of that at 1 m depth (Fig. S2A).

Primary production, total biomass and chlorophyll *a*

Lowest average primary production at the 1 m level was recorded in May (~ 14 µg C l^{-1} d^{-1}), with an increasing trend observed in the following months, reaching ~51 µg C l^{-1} d^{-1} in August (Fig. 2A). Primary production at 5 m was only about 1% of that at 1 m depth (Fig. S 2B). Total biomass of phytoplankton varied on average between 19.7 and 71.8 µg C l^{-1}, with lower values in May than later in the season (Fig. 2B). Temp, salinity and SPM were found to be the main factors influencing primary production, while total biomass was influenced by Tot P, salinity and Tot N (Table 2). The concentrations of Chl *a* were lowest in May (~ 1 µg l^{-1}) and increased 3-fold in June to August (Fig. 2C). Temperature and DOC were indicated to be major drivers of Chl *a* (Table 2).

Phytoplankton primary production and heterotrophic bacterial production constituted the total "basal production". The total production was higher in May than during the summer, mainly due to the peak of heterotrophic bacterial production. The relative importance of primary production showed an increasing trend from spring to summer (Fig. S3). This pattern was observed both at 1 m depth level as well as on the depth integrated data (Fig. S3). Phytoplankton primary production constituted <10% of the basal production in May, while in August they contributed with 40-60% of the production (Fig. S3).

Phytoplankton species composition, size-structure and nutritional strategy

The phytoplankton community composition varied between sampled months (ANOSIM global $R = 0.433$, $p < 0.001$), and was influenced by the river plume. NMDS ordination showed that the phytoplankton community structure was relatively similar in the estuary during the summer, while the community sampled in May was separated from other months. Additionally, the phytoplankton community in the river plume (station: 1, 2, 3 and 4) clearly differed from that at the more seaward stations in the summer months, clustering more closely with the spring/May samples (Fig. 3). Diatoms and dinoflagellates dominated the community in spring, while filamentous cyanobacteria dominated the biomass in the summer (Table 3, Fig. 4A).

The phytoplankton size-structure was dominated by microphytoplankton (> 20 µm) during the entire study period, with biomasses ranging from 1.8 to 133 µg C l^{-1}. The proportion of microphytoplankton was lowest in May, constituting ~50% of the phytoplankton biomass, later increasing to ~70% (Fig. 4B). Both biomass and the relative contribution of microphytoplankton correlated with most of the environmental variables, e.g. increased with increasing Temp, Tot P and decreasing with tDOM related variables, Tot N or Kd (Table S1). In May microphytoplankton was dominated by *Wolozynskia* spp. and *Planktothrix* sp. Filamentous cyanobacteria became dominant members of the community at all post-May sampling events, with a peak of *Planktothrix* spp. and *Aphanizomenon* spp. in August (Table 4). Nanophytoplankton biomass (2-20 µm) varied between 3.6 and 28.1 µg C l^{-1}, and was on average 2-fold lower in May than in August. The highest proportion of nanophytoplankton (2-20 µm) was observed in May, constituting ~32% of the total biomass, with a fairly stable and slightly lower relative contribution recorded in later sampling months (Fig. 4B). Nanophytoplankton was dominated by the genus *Chrysochromulina* during spring and early summer (Table 4). Negative correlations were found between nanophytoplankton biomass and tDOM related variables, Tot N, Kd and water discharge, while a positive correlation was observed in relation to Temp, salinity and pH (Table S1). On the other hand, the proportion of nanoplankton in the total phytoplankton biomass increased with increasing Tot N and water discharge or decreasing Temp, Tot P and pH. Picoplankton (< 2 µm) biomass ranged from 1.5 to 8.9 µg C l^{-1} with a higher contribution of small cells in May and July than June and August (Fig. 4B). The relationship between picocyanobacterial biomass and temperature and salinity was positive, while a negative correlation was found with water discharge. The relative contribution of picocyanobacteria correlated with most of the environmental variables, e.g. they increased with tDOM related variables and decreased with Tot P (Table S1).

The proportion of autotrophic biomass was high throughout the study period, constituting ~85% in May, increasing up to ~89% in July (Fig. 4C). The opposite trend was found for the mixotrophs. The absolute biomass of autotrophs, mixotrophs and heterotrophs correlated with most of the physicochemical variables, while the relative biomass of autotrophs and mixotrophs were influenced by temperature, Tot P and PAR (Table S1). Mixotrophs were dominated by *Chrysochromulina* spp. during the whole study period and Dinophyceae spp. were the most abundant class among heterotrophs.

In the RDA model, the first two axes explained 53.2 % of the variance in the size-structure of the phytoplankton ($p < 0.05$). The first RDA axis was strongly positively correlated with Temp and Tot P and negatively with the relative contribution of mixotrophs (0.56, 0.52, and -0.46, respectively) (Fig. 5, Table S2). It explained the

temporal variability of the size-structure of the phytoplankton during the study period. The second RDA axis explained the spatial variability of the size-structure of the phytoplankton and was strongly correlated with the relative contribution of autotrophs and mixotrophs (-0.38 and 0.34, respectively). Forward selection indicated that temperature and Tot P were the variables statistically significantly shaping the size-structure of the phytoplankton and explained 22.4%, and 11%, respectively, of the total variance (Table 5).

Phytoplankton diversity and resource use efficiency (RUE)

Taxonomic richness was the lowest in May and the highest in July (Fig. 6A). Temp and Tot P were the most important factors influencing taxonomic richness (Table 2). The average RUEp was the lowest in May (~ 0.32) then increased and remained constant for the rest of the period (Fig. 6B). RUEp was shaped by Tot P, Tot N and salinity (Table 2). Furthermore, higher phytoplankton diversity led to more efficient use of phosphorus (Spearman rho = 0.44, $p < 0.001$) (Fig. 6C). SEM analyses showed that both Temp and Tot P had a strong significant impact on richness (respectively, 0.49, $p < 0.001$, 28.37, $p < 0.01$). Moreover RUEp was directly influenced by Temp (0.02, $p < 0.01$) and indirectly by Tot P through richness mediator (0.02, $p < 0.01$) (Fig. 7). Higher Temp and Tot P concentration lead to higher phytoplankton biomass (TB) and primary production (Fig. 7). Overall, SEM models showed a similar goodness of fit to the data (Tot P model: Fisher's $C = 7.0$, $df = 6$, $p = 0.33$, Temp model: Fisher's $C = 7.4$, $df = 6$, $p = 0.29$).

Discussion

Our results indicate that the phytoplankton production, size-structure and nutritional strategy were affected in a complex way by the concurrent effects of factors like temperature, Tot P and tDOM variables. The proportion of small cells picocyanobacteria and nanophytoplankton in the total phytoplankton biomass was negatively correlated with Tot P which can be explained by the increased importance of smaller cells under lower nutrient concentrations, due to higher surface-to-volume ratio (Bell and Kalff, 2001; Callieri et al., 2007). It is likely that re-mineralization of phosphorus was higher in the warm summer than in the cold spring, leading to higher P availability in summer. The absolute biomass of pico- and nanophytoplankton, on the contrary, related positively to temperature, which is in agreement with earlier studies (e.g. Andersson et al., 1994; Moran et al., 2010). Species with small cell size, such as *Synechococcus* spp., in general have higher specific growth rates at high temperature (Jöhnk et al., 2008; Paerl and Huisman, 2009). A previous study performed at a coastal location in the northern Baltic Sea, estimated the generation time of picocyanobacteria to be a few days under summer conditions, while it increased to ~120 days during winter (Andersson et al., 1994). Temperature can thus be a direct driver of phytoplankton community composition, however due to a strong covariance with nutrient concentrations, individual effect can often be difficult to distinguish (Li, 1998; Agawin et al., 2000; Mousing et al., 2014). During summer samplings (June-August), lower river discharge impacted only minimally on the majority of estuarine stations, and the effective light climate and temperature were relatively high across the majority of the estuarine stations. This is in agreement with studies from shallow humic lakes where temperature and light climate

were determined to be the main factors limiting small cells abundance (Jasser and Arvola, 2003).

The proportion of nanophytoplankton correlated positively with water discharge and Tot N. The nanophytoplankton fraction was dominated by the mixotrophic flagellate *Chrysochromulina* spp. during most of the study period. Mixotrophs combine photosynthesis and phagotrophy, which comes with associated metabolic costs, leading to lower reproductive rates compared to single nutritional mode organisms (Rothhaupt, 1996). This means that mixotrophs have a competitive advantage in environments where nutrient concentrations are low, light availability limited and where they can gain nutrients via consumption of bacteria (Hajdu et al., 1996; Jansson et al., 1996; Dahl et al., 2005). Heterotrophic bacteria were promoted by the spring flush and inputs of bioavailable carbon (Figueroa et al., 2016), which in turn promoted the mixotrophs, potentially important mediators of bacterivory in coastal waters (Havskum and Riemann, 1996), likely feeding mixotrophically to supplement the constrained availability of nutrients such as P (Nygaard and Tobiesen, 1993; Jansson et al., 1996). The important role of mixotrophy in our study system was further cemented by the positive relationship between mixotrophs and bacterial production, which in turn was influenced by tDOM. Similar relationships have been observed in humic lakes (Drakare et al., 2002; Bergström et al., 2003), indicating that phytoplankton communities in subarctic estuaries are regulated in a similar way as unproductive humic lakes.

The proportion of microphytoplankton increased with higher Tot P concentrations and higher temperature. Diatoms dominated the phytoplankton community during the spring, likely due to water turbulence, high suspended matter load and the high Tot N:P ratio (Margalef, 1978; Kiørboe, 1993). However, we did not observe a spring phytoplankton bloom, probably due to high freshwater inflows which have been shown to counteract spring blooms in shallow coastal systems (Gasiūnaitė et al., 2005). A clear shift from diatoms to filamentous cyanobacteria was observed during the summer, as a consequence of changes in Tot N:P ratio, tDOM related variables and increasing temperature and salinity. This is likely explained by adaptation of large filamentous cyanobacteria to high temperature in combination with a capability to store phosphorous within the cells, nitrogen fixation and buoyant regulation via gas vacuoles. Cyanobacteria generally have growth optima at relatively high temperature, giving them a competitive advantage over diatoms during warmer summer months (Jöhnk et al., 2008). From May to July, conditions favored Oscillatoriales, which in the Baltic Sea comprises non-nitrogen fixing filamentous forms. In August, their contribution decreased while the abundance of Nostocales increased, reaching ~50%. This can be explained by the lower N:P ratio which promoted filamentous cyanobacteria capable of atmospheric nitrogen fixation (*Aphanizomenon* spp. and *Dolichospermum* spp.). Additionally, a positive relationship between cyanobacteria and salinity was found, supporting the idea that salinity can play an important role in shaping the cyanobacterial community (Andersson et al., 2015).

In estuaries, freshwater inflows can play an important role in regulating the balance between bacterial and primary production due to the transport of nutrients and carbon, and the influence on light availability (Hoch and Kirchman, 1993). The primary production to bacterial production ratio was < 1 in May which indicates that the ecosystem was net heterotrophic, while it switched to net autotrophy by late summer (August). Bacterial production was positively correlated with tDOM related parameters, suggesting that allochthonous carbon carried by water discharge promoted bacterial growth and decoupled

474 them from primary production in the spring while autochthonous produced carbon was the
475 main source of carbon during summer.
476
477 According to climate change scenarios the precipitation will increase ~30 % in the
478 northern Baltic Sea region, causing increased land runoff of tDOM, and the temperature
479 will rise ~4□ during the next coming century (Andersson et al. 2015). Ecological
480 consequences may be lowered primary production in the northern Baltic Sea basins due to
481 brownification of the seawater, and increased heterotrophic bacterial production due to
482 higher inflow of tDOM and elevated temperature (Andersson et al. 2015). As a result,
483 ecosystem can shift from net autotrophy to net heterotrophy. The energy transfer up the
484 food web may decrease and the biomass of higher trophic level reduced. Additionally, the
485 composition of phytoplankton and heterotrophic bacteria will probably change.
486 Abundances of mixotrophs may increase and bacterial taxa degrading high molecular
487 weight compounds become common.
488
489 The increased primary production from spring to summer seems to have been mediated
490 by higher temperatures and higher Tot P concentrations. tDOM concentration was
491 probably not a major influence on primary production, since average PAR values at 1 m
492 depth were always at saturating levels for primary production, i.e. > 70 µmol quanta m^{-2} s^{-1}
493 (Andersson et al., 1994). At 5 m depth however, the PAR values were low (<10 µmol
494 quanta m^{-2} s^{-1}) and therefore also the primary production rates were minor, indicating that
495 efficient primary production only occurred in a relatively small and distinct portion of the
496 water column.

497 Our study indicates that higher temperature and phosphorus concentrations led to
498 increased taxonomic richness, which in turn promoted resource use efficiency and high
499 primary production. This is consistent with previous studies, performed on relatively small
500 area (e.g. less than 10 km) and within a short period of time, where a positive or unimodal
501 diversity-productivity relationship has been found (Chase and Leibold, 2002; Korhonen et
502 al., 2011). The observed trends in community composition across the sampling period and
503 the complex interactions between physicochemical and biological factors are likely
504 indicative of selection processes and complementarity effects (Loreau and Hector, 2001).
505 Additionally, higher phytoplankton diversity led to more efficient use of phosphorus. It
506 confirmed that a more diverse community is able to capture limiting nutrient more
507 efficiently, and higher overall productivity is the result.

508 In conclusion, our study show that primary production, the phytoplankton size-structure,
509 and the phytoplankton nutritional strategy in phosphorus-poor estuaries receiving
510 terrestrial input follow patterns more reminiscent of humic lakes than those observed in the
511 open sea. Elevated levels of tDOM related variables and low concentrations of phosphorus
512 favored smaller cell-sizes due to their higher surface to volume ratio and higher light
513 harvesting efficiency. Furthermore, the relative contribution of mixotrophs was higher
514 when basal production was dominated by bacteria, supporting observations that grazing of
515 bacterioplankton can be an important nutrient source under environmental conditions
516 generally perceived as unfavourable or limiting. The decreasing Tot N:P ratio was found to
517 be a main factor shaping changes in the community composition of filamentous
518 cyanobacteria, shifting the community towards nitrogen-fixing species during summer. A
519 positive relationship between phytoplankton taxonomic richness, resource use efficiency
520 and productivity were found. Furthermore, temperature seems to be a dominant factor, as
521 can be expected in regions with high seasonality. Climate change induced increased
522 temperature might therefore lead to increased resource use efficiency and in turn high

primary production in shallow coastal areas. On the other hand, browning of the water points towards decreased primary production and a stronger reliance on heterotrophic processes. Both such processes can have potential ecosystem impacts, such as oxygen depletion, an increase in release of greenhouses gases to the atmosphere or a decrease in food quality transfer to higher trophic levels. However, the net effect on primary production and wider ecosystem function is presently difficult to interpret and would need modelling studies.

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Author contribution

Joanna Paczkowska (JP), Owen Rowe (OR), Daniela Figueroa (DF) and Agneta Andersson (AA) jointly designed the study, performed the field work and analyzed the samples. JP performed the statistical analyses and wrote the article together with AA. OR and DF commented on the written text.

812 **Table 1.** Monthly mean (± standard deviation) of physicochemical variables for all
813 sampled stations during the study period.

	May	June	July	August

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Temp (°C)	6.7 ± 0.7	15.7 ± 0.7	21.4 ± 1.0	16.5 ± 0.5
Salinity	0.3 ± 0.3	0.6 ± 0.5	0.5 ± 0.4	1.0 ± 0.6
Tot N (µmol l^{-1})	26.9 ± 2.9	19.7 ± 2.8	20.8 ± 3.0	20.4 ± 2.3
Tot P (µmol l^{-1})	0.29	0.32 ± 0.1	0.32	0.34 ± 0.1
N:P ratio	92 ± 9.4	63.3 ± 11.4	64.6 ± 10.4	62.4 ± 9.6
HS (µg l^{-1})	61.5 ± 8.2	43.8 ± 10.5	53.6 ± 13.1	41.9 ± 12.5
DOC (mg l^{-1})	7.6 ± 1.6	5.6 ± 0.7	6.3 ± 0.7	6.6 ± 3.3
CDOM (m^{-1})	3.0 ± 0.6	2.8 ± 0.9	2.9 ± 0.8	2.1 ± 0.8
SPM (g m^{-3})	3.2 ± 1.2	3.4 ± 1.5	3.5 ± 1.7	3.7 ± 2.9
Kd (m^{-1})	1.8 ± 0.3	1.2 ± 0.3	1.3 ± 0.4	1.2 ± 0.5
PAR at 1m (µmol photon m^{-2} s^{-1})	101± 62	40 ± 25	169 ± 125	104 ± 84
PAR at 5m (µmol photon m^{-2} s^{-1})	3 ± 5	1.7 ± 1.7	7 ± 4	7 ± 8
River discharge (m^3 s^{-1})	99.3 ± 3.0	33.4 ± 1.4	33.1 ± 1.1	24.6 ± 0.9
pH	6.9 ± 0.1	7.2 ± 0.3	7.2 ± 0.2	7.4 ± 0.2

Table 2. Results of generalized linear models (GLMM) on the physicochemical variables influencing phytoplankton variables during the study period (AIC: Akaike Information Criterion). Phytoplankton variables: primary production (PP), total phytoplankton biomass (TB), chlorophyll *a* (Chl *a*), taxonomic richness (S) and resource use efficiency (RUE_p).

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		Estimate	SD	t-value	p
PP	Temp	0.067	0.016	4.282	<0.001
AIC 535.16	Salinity	1.223	0.213	5.746	<0.001
	SPM	0.124	0.038	3.267	<0.01
TB	Tot P	5.690	0.835	6.817	<0.001
AIC 549.02	Salinity	0.399	0.126	3.183	<0.01
	Tot N	-0.062	0.020	-3.026	<0.01
Chl *a*	Temp	0.179	0.017	10.336	<0.001
AIC 136.07	DOC	-0.215	0.087	-2.488	<0.05
S	Tot P	1.012	0.260	3.886	<0.001
AIC 366.44	Temp	0.011	0.004	2.921	<0.01
	Salinity	0.152	0.058	2.609	<0.05
	CDOM	0.084	0.041	2.073	<0.05
RUEp	Tot P	2.410	0.340	7.106	<0.001
AIC -58.41	Salinity	0.183	0.046	3.948	<0.001
	Tot N	-0.036	0.006	-5.903	<0.001

838

839 **Table 3.** Monthly mean relative carbon biomass (%) of different phytoplankton groups in
840 the study area (May-August).

841

Class/group	May	June	July	August
Cyanophyceae*	6.7	46.5	52.7	60.1
Picocyanobacteria	15.8	3.8	11.1	6.8
Dinophyceae	16.6	3.9	1.2	0.6
Diatomophyceae	32.3	25.4	18.0	13.2
Prymnesiophyceae	11.2	9.7	5.0	3.7
Others	17.5	10.8	11.9	15.5

842 * Colony-forming and filamentous cyanobacteria

843

844 **Table 4.** Dominant phytoplankton taxa in different size classes for 19 stations (constituting
845 >25% of the total carbon biomass), in the study area (May-August).

846

Size fraction	Class/Phylum	May	June	July	August
< 2 µm	Cyanophyceae	*Synechococcus* spp.	*Synechococcus* spp.	*Synechococcus* spp.	*Synechococcus* spp.
2-20 µm	Prymnesiophyceae	*Chrysochromulina* spp.	*Chrysochromulina* spp.	*Chrysochromulina spp.*	
> 20 µm	Cyanophyceae		*Planktothrix* spp.	*Planktothrix* spp.	*Planktothrix* spp. *Aphanizomenon* spp.

847

848

849

850 **Table 5.** Results of the forward selection of physicochemical and biological variables that
851 significantly influenced the size-structure of phytoplankton during the study period.

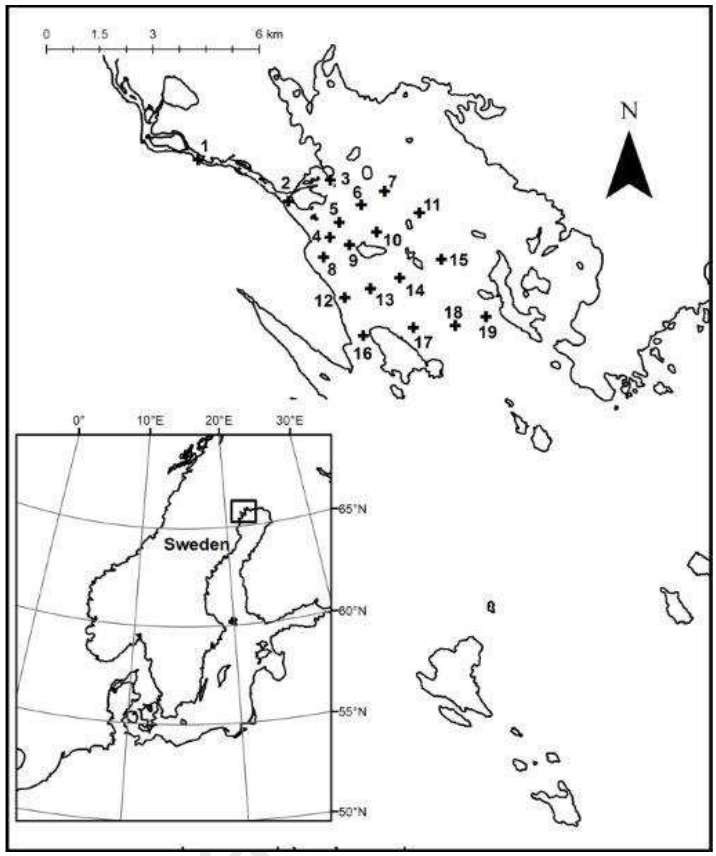
852

Variables	% explained	p-value	*F*-value
Temp	22.4	0.015	18.5
Tot P	11.0	0.015	10.4

853
854

855 **Figure 1.**

856



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859 **Figure 1.** Map of the study area, northern Baltic Sea, indicating the stations sampled (from
860 Figueroa et al., 2016).

861
862

Figure 2.

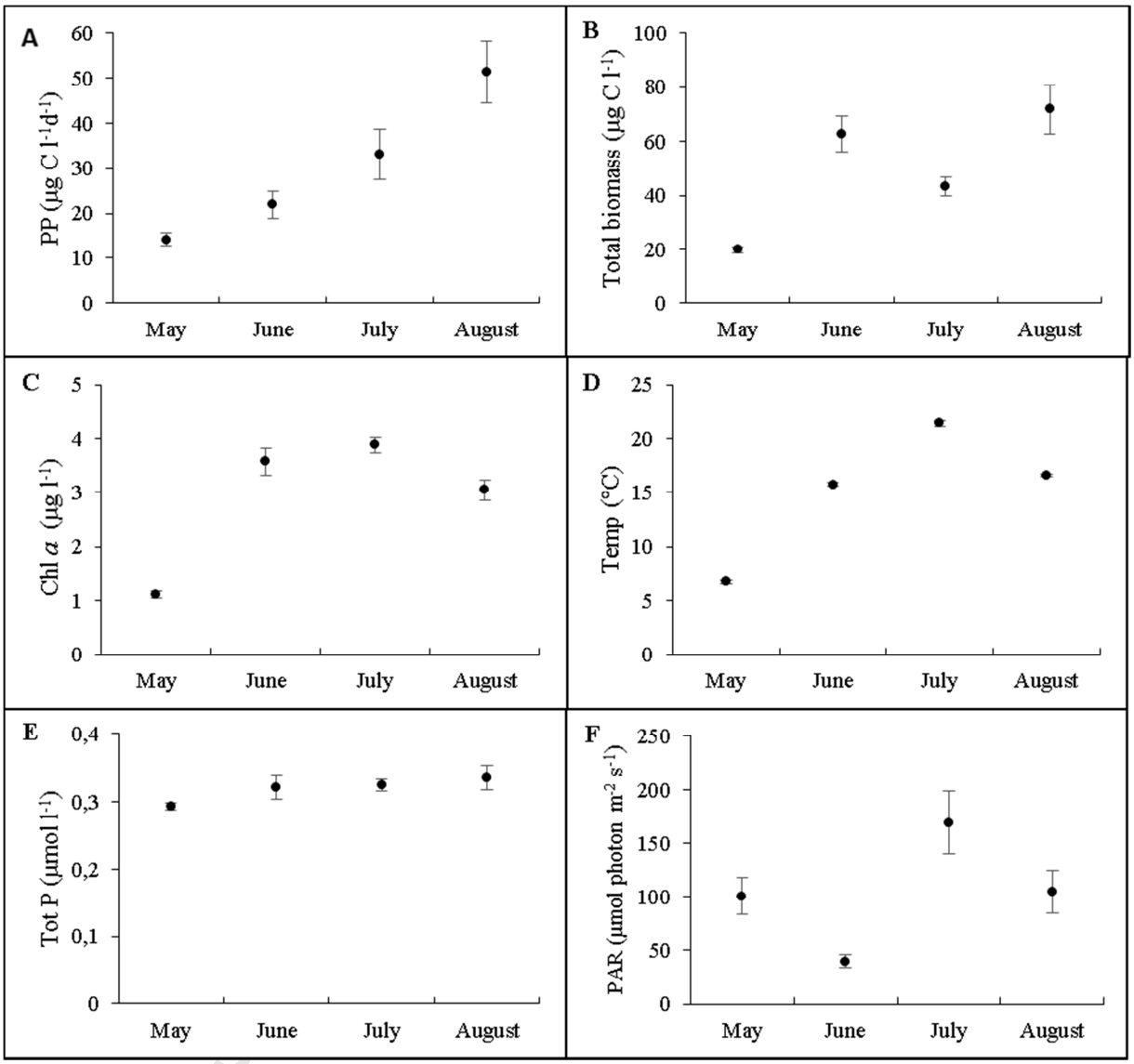
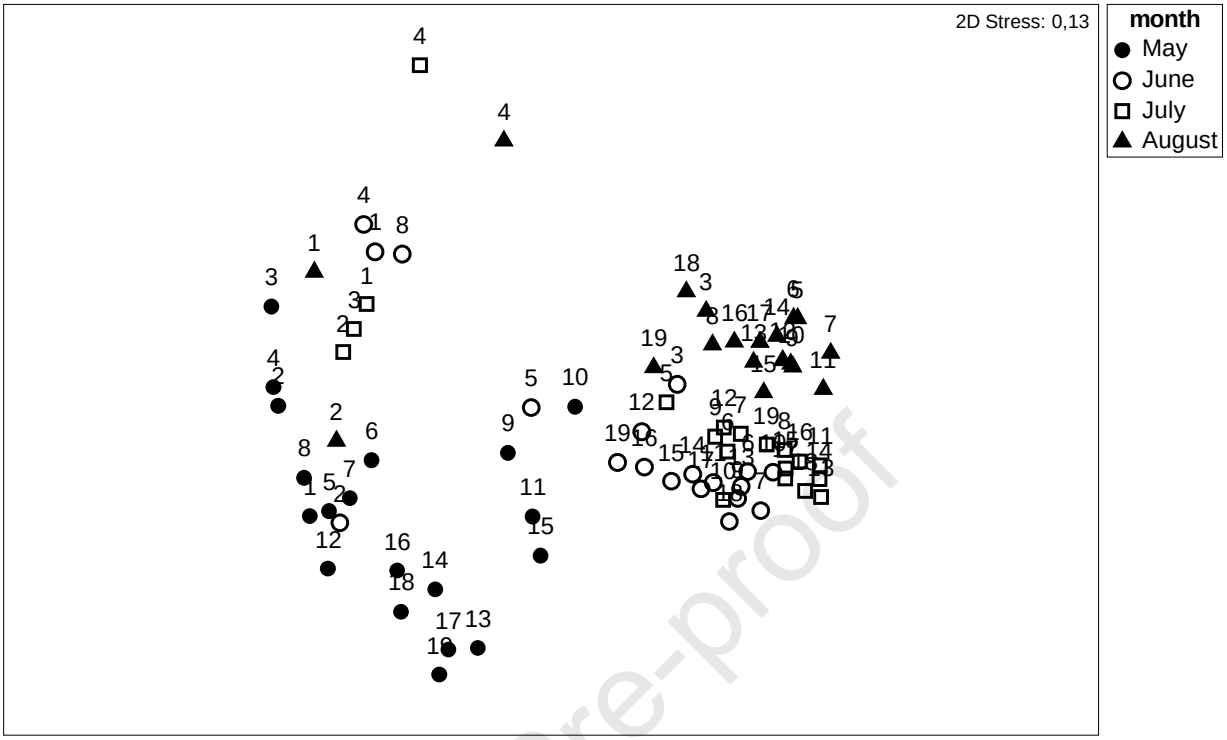


Figure 2. Monthly average primary production (A), total phytoplankton biomass (B) chlorophyll *a* (C), temperature (D), total phosphorous (E) and average PAR at 1 m depth (F) in the study area, May-August 2011. Error bars denote standard error.

876 **Figure 3.**

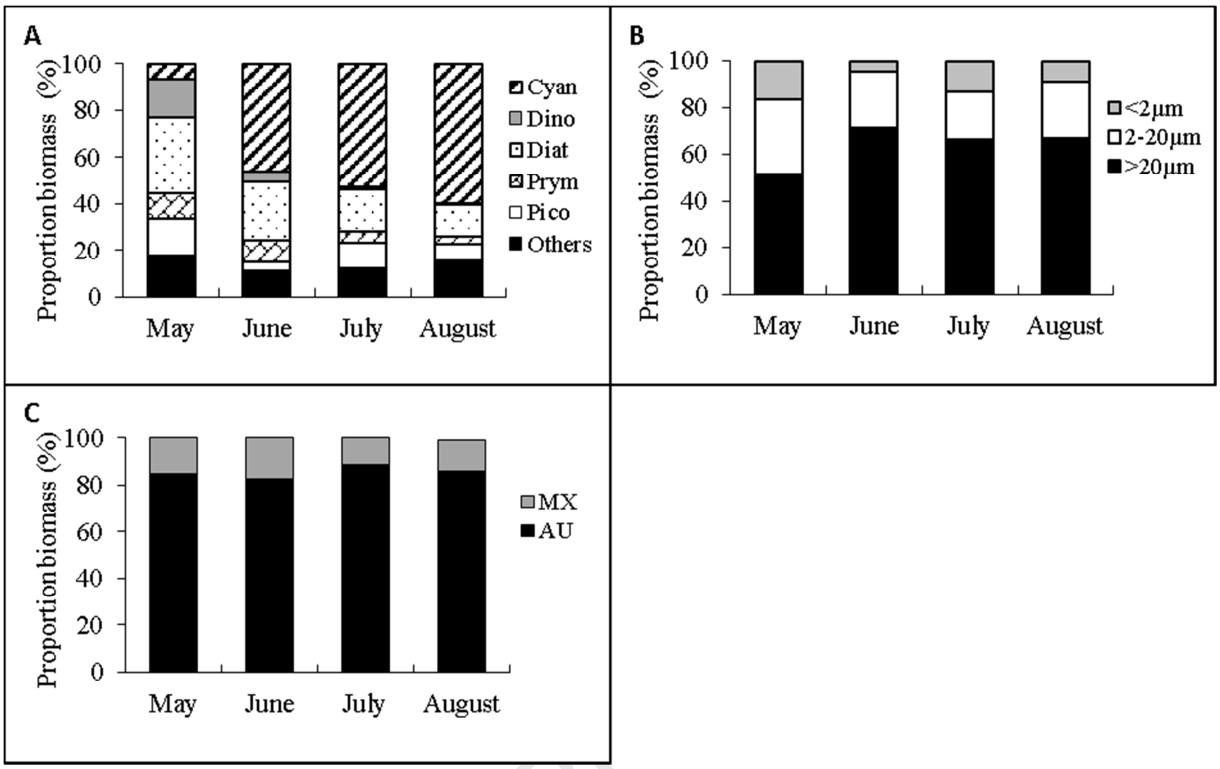


877

878 **Figure 3.** Non-metric multidimensional scaling (NMDS) of the phytoplankton community
879 in the study area, May-August 2011. Numbers represent different sampling stations, with
880 station 1 being the river and 2 the river mouth (see Fig. 1).

881

882 **Figure 4**



883

884

885 **Figure 4.** Monthly average relative phytoplankton biomass (%) of phytoplankton groups:
886 Cyanophyceae (Cyan), Dinophyceae (Dino), Diatomophyceae (Diat), Prymnesiophyceae
887 (Prym), Picocyanobacteria (Pico) and Others (A), size groups pico- (< 2 µm),
888 nano- (2-20 µm), micro- (> 20 µm) phytoplankton (B), and AU (autotrophs) and MX
889 (mixotrophs) (C) in the study area, May-August 2011.

890

Figure 5.

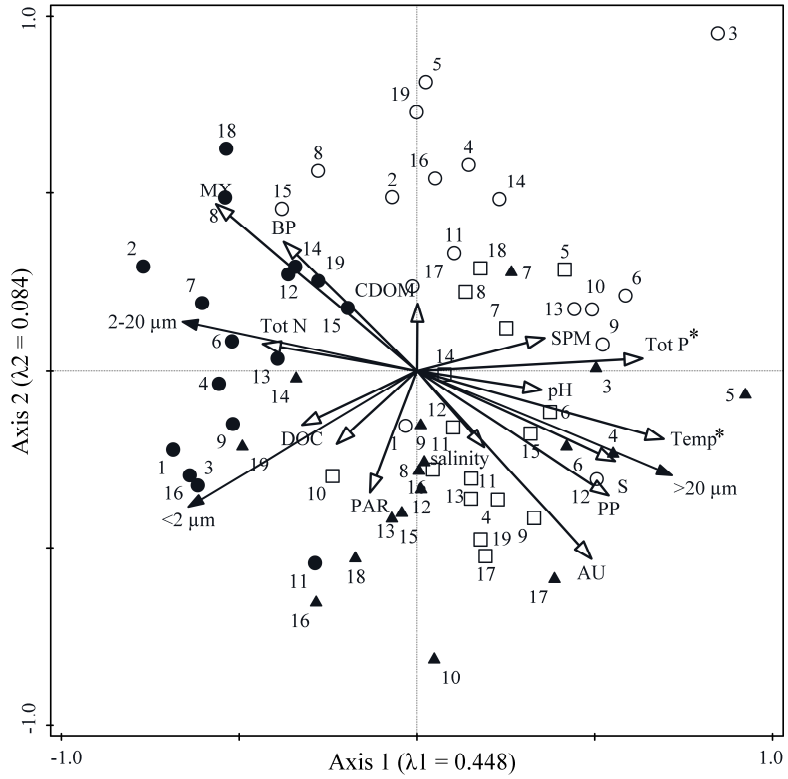
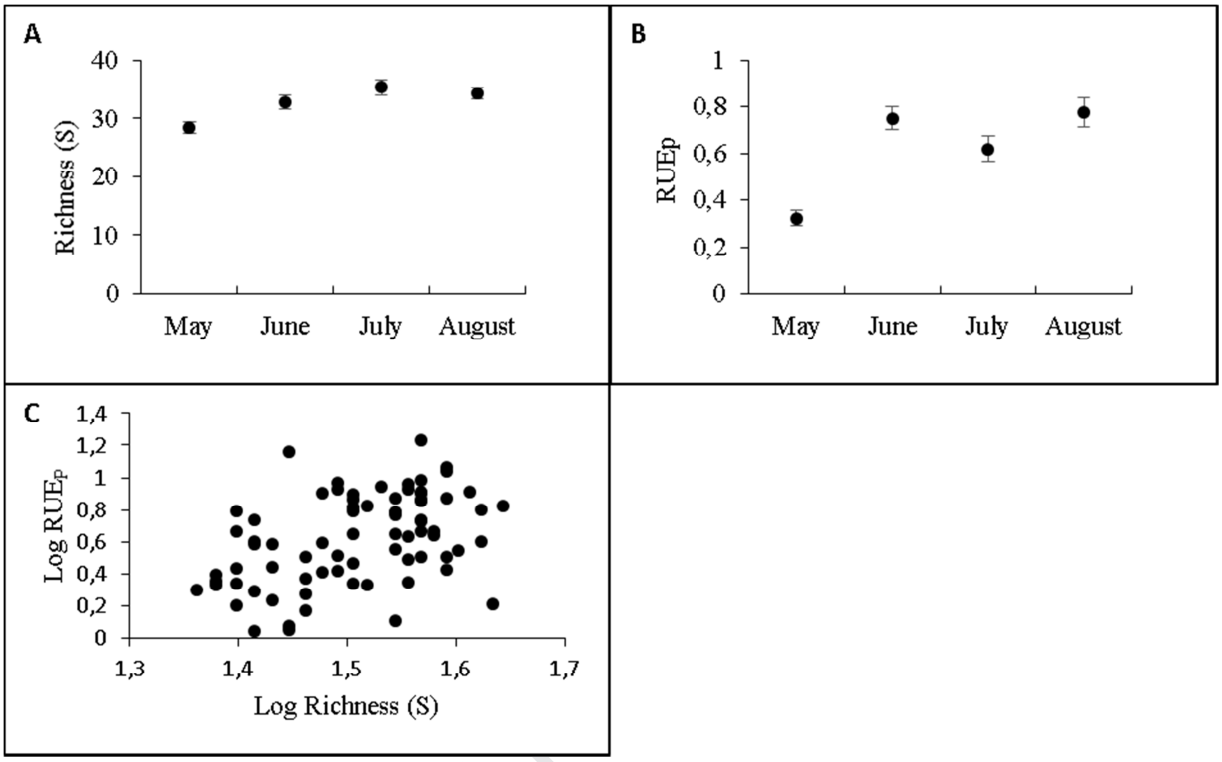


Figure 5. Redundancy analysis (RDA) ordination plot of the relative contribution of phytoplankton size-structure: pico- (< 2 µm), nano- (2-20 µm), micro- (> 20 µm) phytoplankton, and physicochemical (DOC, CDOM, Tot P, Tot N, Temp, PAR at 1m, SPM, salinity, pH) and biological (primary production (PP), bacterial production (BP), the relative biomass of autotrophs and mixotrophs (AU, MX), taxonomic richness (S) variables during the study period (●- May; ○ – June, □ – July, ▲- August). Asterisks indicate statistical significance ($p < 0.05$) physiochemical variables influence the relative contribution of phytoplankton size-structure based on RDA, forward selection method.

911

912 **Figure 6.**



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915

916 **Figure 6.** Monthly average taxonomic richness (A), resource use efficiency (B), and the
917 relationship resource use efficiency vs. taxonomic richness (C) in the study area, May-
918 August 2011. Error bars denote standard error.

919

Figure 7.

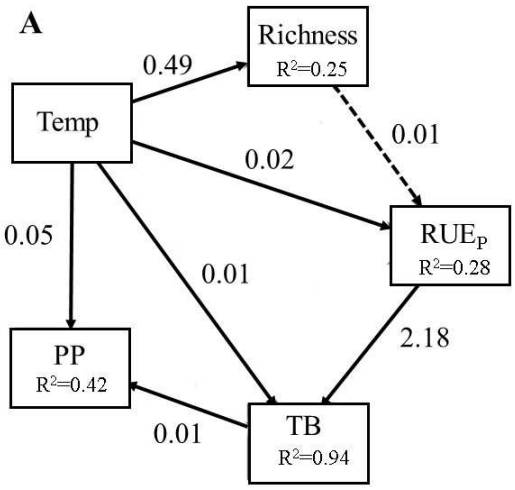

Figure 7. Path diagram for structural equation model relating temperature, Temp (A), and total phosphorus, Tot P (B), to taxonomic richness, resource use efficiency (RUEp), total phytoplankton biomass (TB) and primary production (PP). The numbers next to each arrow are unstandardized regression coefficients of the SEM. Solid black arrows represent significant paths ($p < 0.05$) while dash arrows non-significant paths ($p > 0.05$).

Highlights:

- Mixotrophic nanophytoplankton is influenced by spring river flush and bacterial production.
- Phytoplankton diversity and resource use efficiency are promoted by summer warming and phosphorus concentration.
- High resource use efficiency sustains phytoplankton biomass and primary production.
- Autotrophic microphytoplankton is favored during the summer primary production maximum.